

PROJECT NUMBER: 1902  
PROJECT TITLE: Tobacco Microbiology  
PROJECT LEADER: D. J. Ayers  
WRITTEN BY: D. K. Chadick  
PERIOD COVERED: September, 1988

## I. ART FILLER STORAGE STUDY

- A. Objective: To determine the effects of storage on the microbial load of ART filler material at 35°C and 25°C with 80% RH.
- B. Status: To date, four of the five experiments have been initiated in this study.
- C. Results: Bacterial counts showed only a slight decrease or no change over initial counts for the data accumulated from experiments 1-4 after 4, 2, 2, and 1 week(s) of storage, respectively. Mold and yeast counts were variable and exceeded acceptable laboratory limits ( $\leq 80$  mold and/or yeast colonies per gram, see Reference 1) in all four experiments to date (2).
- D. Plans: Complete study.
- E. References:
1. Crockett, E. A. The Microbial Quality Improvement Program (MQIP) As Conducted in the OC Semi-Works Primary Facility. Special Report #87-115; 1987 December 21.
  2. Jones, J. Notebook No. 8590, pp. 73, 75, 77-80.

## II. BACTOMETER SCATTER EXPERIMENTS

- A. Objective: To determine the normal scatter in detection times of mixed and pure bacterial cultures (1).
- B. Results: In an effort to determine if possible differences in cellular growth phase between previously conducted experiments might be responsible for an observed variation (2), the protocol was revised to utilize cells in the log phase of growth. The results of duplicate experiments continued to demonstrate high variation between experiments when using either a pure culture of Bacillus subtilis and B. pumilus or a mixed culture of B. subtilis, B. pumilus and B. circulans. However, the variation was reduced significantly between duplicate experiments using log phase B. circulans as a pure culture (3).
- C. Plans: Investigate effect of spores on detection times.
- D. References:

1. Ayers D.J. Notebook No. 8210, pp. 161-166.
2. Ayers, D.J. Project 1902 Monthly Summary. PM Redbook Accession Number 88-052; 1988 August 15.
3. Gaines, O. Notebook No. 8690, pp. 27-36, 38-42.

### III. BACTOMETER CALIBRATION CURVES

- A. Objective: To generate calibration curves for determining the number of bacteria present in a sample using the Bactometer.
- B. Status: Data (bacterial counts and detection times) have been collected from five sampling periods each of Bright, Burley, and Oriental Tobacco, and RLTC, RL150B, and a Marlboro Blend material. The data have been submitted to J. Tindall for statistical analyses.
- C. Plans: Further efforts in this area await analysis by J. Tindall.
- D. Reference:
  1. Chadick, D. Notebook No. 8625, pp. 96-98, 101-102, 104-107, 110-111, 113-114, 116-119, 125-132, 139-140, 143-144.

### IV. BACTOMETER ORIENTAL EXTRACT STUDY

- A. Objective: To determine if concentrated Oriental tobacco extract contains antimicrobial factors that are affecting Bactometer detection times (1).
- B. Results: In addition to the previously mentioned experiments (2), three experiments were conducted to determine the effect of a concentrated Oriental extract (100x) on the growth of B. pumilus. The detection times were longer from the culture diluted in the Oriental extract when compared to the times obtained from the same culture diluted in saline. These new results agreed favorably with the previously reported results (2,3).
- C. Plans: A memo will be issued.
- D. References:
  1. Jones, J. Notebook No. 8590, pp. 70-71.
  2. Ayers, D. Project 1902 Monthly Summary. PM Redbook Accession Number 88-052; 1988 August 15.
  3. Jones, J. Notebook No. 8590, pp. 66, 69-72, 76.

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